

# DNA Adducts in Human Urinary Bladder and Other Tissues

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Tobacco smoking is associated with an increased risk of cancer in a number of organs, including bladder and lung. Tobacco smoke contains at least 50 known chemical carcinogens that exert their biological effects through their covalent binding to cellular DNA. Examining human DNA for the presence of altered nucleotides is a means of monitoring exposure to genotoxic chemicals. DNA isolated from 73 human bladder biopsies has been analyzed by <sup>32</sup>P-postlabeling for the presence of aromatic/hydrophobic adducts. Butanol extraction of DNA digests resulted in up to a 3-fold greater recovery of adducts than nuclease P<sub>1</sub> digestion. Among 16 nonsmokers, adduct levels were in the range 3.2-20.8/10<sup>8</sup> nucleotides (mean 9.7). Eight ex-smokers had values in the range 2.6-12.3 (mean 7.1). Thirteen smokers had adduct levels between 1.3 and 26.7 adducts/10<sup>8</sup> nucleotides (mean 9.5, not different from nonsmokers). Six cigar smokers had higher levels of adducts (mean 12.1, range 7.3-15.0), but pipe smokers did not (five samples, mean 8.6, range 2.9-12.7). A further 8 samples from nonsmokers and 17 from smokers were examined in more detail. Although most of the DNA binding appears not to be smoking related, the levels of one adduct were found to be on average 2-fold higher in smokers ( $p < 0.005$ , one-tailed  $t$  test). Studies on tissues of the respiratory tract demonstrate a correlation between DNA adduct levels and exposure to tobacco smoke. Evidence to date on the influence of smoking on adducts in peripheral blood cells is equivocal; some studies demonstrate a significant effect, whereas others do not. It is probable that the effect of smoking on adduct formation on blood cells is a weak one and that other undetermined exposures have greater influence.

## Introduction

The risk to human health of cigarette smoking has been extensively studied and documented. Although the major site of cancer induction by tobacco smoke is the lung, smoking is also an important cause of bladder cancer in humans (1). In countries in which tobacco smoking is a widespread and long-standing habit, around half of all cases of bladder cancer in men, and around a quarter of cases in women, are attributable to cigarette smoking. Pipe and cigar smoking probably also constitute an increase to the risk of developing bladder cancer, but at a lower level of risk than that associated with cigarette smoking.

Tobacco smoke is a complex mixture of several thousand identified chemicals, including more than 50 that are carcinogenic to experimental animals. Three major classes of carcinogens that are found are nitrosamines, polycyclic aromatic hydrocarbons (PAHs), and aromatic amines. DNA adducts formed by the latter two classes can be readily detected by <sup>32</sup>P-postlabeling analysis (2). Two variations on the procedure that dramatically enhance its sensitivity can be applied to the detection of some classes of adducts: the butanol extraction method will detect adducts formed by PAHs and aromatic amines, whereas the nuclease P<sub>1</sub> digestion procedure detects PAH-DNA adducts, but not, under some conditions, those formed by aromatic amines

(3,4). Thus, comparative use of these procedures can reveal qualitative information about the nature of adducts detected in human tissues.

In this paper we describe results of experiments to investigate the influence of tobacco smoking on the levels of DNA adducts in human urinary bladder epithelium and compare these findings with results of studies of other human tissues, notably cells of the respiratory tract and circulating blood cells.

## Materials and Methods

Biopsy samples of bladder epithelium were taken from 73 individuals undergoing cystoscopic examinations. The samples were frozen and stored at -75°C before DNA isolation. DNA was isolated by a solvent extraction procedure adapted for small tissue samples, as described by Schoket et al. (5).

DNA samples (4 µg) were digested enzymically to 3'-monophosphates with micrococcal nuclease and spleen phosphodiesterase (2) and then either further digested with nuclease P<sub>1</sub> (6) or extracted with butanol (7). The DNA digests or the residues of the butanol extracts were then <sup>32</sup>P-labeled by incubation with carrier-free [ $\gamma$ -<sup>32</sup>P]ATP (50 µCi, 1.85 MBq) and T4 polynucleotide kinase, and the reaction terminated by the addition of potato apyrase (3). Resolution of the labeled adducts was then carried out on polyethyleneimine-cellulose (PEI-cellulose) TLC sheets using the solvents used in earlier studies (8): D1, 1 M sodium phosphate, pH 6.0, on to a filter-paper wick; D2 (opposite to D1), 3.5 M lithium formate, 8.5 M urea, pH 3.5; D3 (at 90° to D2), 0.8 M LiCl, 0.5 M Tris-HCl, 8.5 M urea, pH 8.0; D4 (same direction as D3), 1.7 M sodium phosphate, pH 6.0,

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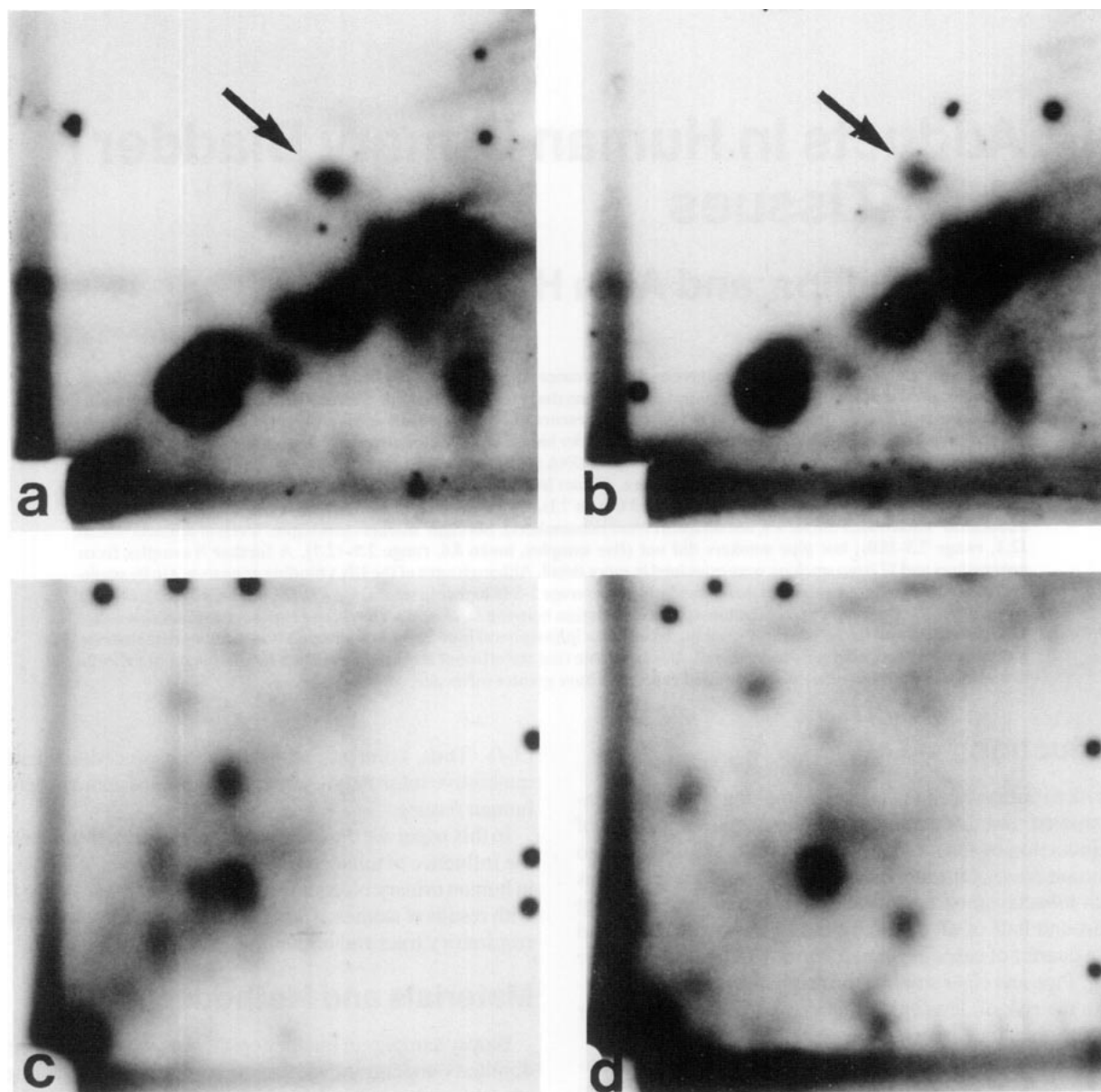


FIGURE 1. Examples of autoradiographs of PEI-cellulose chromatograms of  $^{32}\text{P}$ -postlabeled adducts in human bladder DNA. (a) Butanol-extraction-enhanced labeling of DNA from a 71-year-old male smoker (10 cigarettes/day); (b) butanol-extraction-enhanced labeling of DNA from a 91-year-old female nonsmoker; (c) nuclease  $\text{P}_1$ -enhanced labeling of DNA from a 69-year-old male smoker (20 cigarettes/day); (d) nuclease  $\text{P}_1$ -enhanced labeling of DNA from a 66-year-old female nonsmoker. Adduct 5 (butanol extraction) is indicated by an arrow.

on to a filter-paper wick. Adducts were detected by autoradiography and quantitated by Cerenkov counting of the appropriate areas of the chromatograms.

## DNA Adducts in Human Urinary Bladder

Samples from 48 individuals were analyzed by  $^{32}\text{P}$ -postlabeling using the butanol extraction and nuclease  $\text{P}_1$  digestion enhance-

ment procedures. Examples of the chromatograms of  $^{32}\text{P}$ -labeled adducts obtained are shown in Figure 1. All samples exhibited a complex pattern of adduct spots and areas of unresolved radioactivity. There were no readily apparent qualitative differences between the profiles of DNA adducts from smokers and nonsmokers. A 3- to 5-fold greater recovery of adducts was obtained with the butanol extraction method than when nuclease  $\text{P}_1$  digestion was used. The adduct levels obtained in experiments using butanol extraction are shown in Figure 2. Among 16 non-

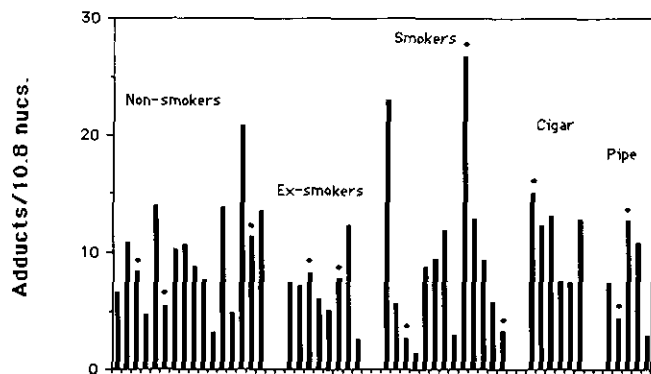


FIGURE 2. Total adduct levels in human bladder DNA samples. Those marked with a diamond (♦) were from patients with a bladder carcinoma or with a past history of carcinoma.

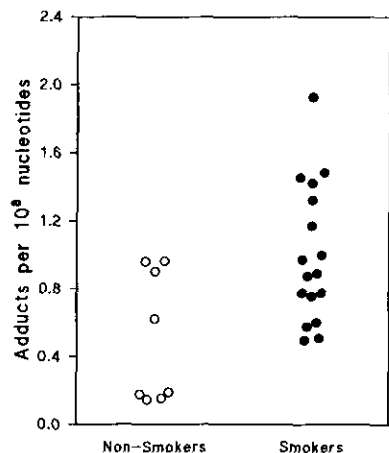


FIGURE 3. Levels of adduct 5 (see Fig. 1) in bladder DNA samples. The mean value for smokers (1.00) was significantly higher ( $p < 0.005$ ) than the mean for nonsmokers (0.51).

smokers, adduct levels were in the range 3.2–20.8/ $10^8$  nucleotides, with a mean value of 9.7. Eight ex-smokers had values in the range 2.6–12.3 (mean 7.1). Thirteen smokers had adduct levels between 1.3 and 26.7/ $10^8$  nucleotides (mean 9.5). Thus, there was no significant increase in levels among cigarette smokers compared to nonsmokers or ex-smokers. Six cigar smokers had between 7.3 and 15.0 adducts/ $10^8$  nucleotides and showed a higher mean value (12.1), but pipe smokers did not (five samples, mean 8.6, range 2.9–12.7). No difference was found between adduct levels in individuals with bladder carcinoma (past or present) and levels in patients without tumors.

A further 8 DNA samples from nonsmokers and 17 samples from smokers were analyzed by  $^{32}\text{P}$ -postlabeling in duplicate using the butanol-extraction enhancement procedure. Similar adduct profiles to those shown in Figure 1 were obtained. No significant differences were found in overall adduct levels between smokers ( $22.7 \pm 8.7$  adducts/ $10^8$  nucleotides; range 13.0–32.8) and nonsmokers ( $17.5 \pm 9.8$  adducts/ $10^8$  nucleotides; range 4.1–36.6). The five major adduct spots were individually quantitated, and comparison of the levels of the individual adducts 1–4 also revealed no differences between smokers and nonsmokers. However, adduct 5 (Fig. 1), accounting for between 2.5 and 6% of the total DNA binding, was 2-fold higher in smokers than nonsmokers (Fig. 3), and this difference

is statistically significant (Student's  $t$  test, one-tailed,  $p < 0.005$ ).

These results are in close agreement with the recently published findings of Talaska et al. (9), who detected an adduct, identified as being derived from 4-aminobiphenyl, at 3- to 4-fold higher levels in bladder DNA from smokers than from non-smokers. However, the chromatographic mobility of adduct 5 reported here is different from that of  $N$ -(deoxyguanosin-8-yl)-4-aminobiphenyl-3',5'-bisphosphate, and its identity has yet to be determined.

The greater levels of adducts detected by the butanol extraction procedure indicate the probable greater contribution to DNA binding of aromatic amines relative to the PAHs, since the levels determined by nuclease  $\text{P}_1$  digestion may be postulated to approximate the contribution to DNA damage by PAH, whereas the levels determined by butanol extraction should include adducts formed both by PAHs and aromatic amines. Thus, these results, in agreement with those of Talaska et al. (9), indicate that the majority of DNA adducts in human bladder derive from sources other than tobacco smoke. Nevertheless, the levels of a relatively minor adduct are significantly elevated in DNA samples from smokers.

## DNA Adducts in Tissues of the Respiratory Tract

In contrast to the results obtained with human bladder DNA, evidence to date suggests that there is a clear association between cigarette smoking and total levels of DNA adducts in cells of the human respiratory tract. In a study of DNA isolated from peripheral lung, a linear correlation was found between daily cigarette consumption and adduct levels (10). The adduct patterns were characteristic of DNA adducts, and a subsequent study of DNA from bronchial epithelium confirmed that the same relationship between adduct levels and smoke intake held for this tissue also (11). Furthermore, similar levels of adducts were determined by both the butanol extraction method and by the nuclease  $\text{P}_1$  method, suggesting that PAHs are the major contributors to DNA binding, with little or no contribution from aromatic amines in this tissue. Other parallel or subsequent studies have arrived at the same conclusion: there is a linear correlation between the levels of DNA adducts in human bronchus and peripheral lung and tobacco smoke intake (12–14). In a study designed to examine a more readily obtainable source of DNA from the human respiratory tract, we have also analyzed DNA from bronchoalveolar lavage cells and have found that adduct levels are significantly higher in cells from smokers than in cells from nonsmokers and ex-smokers (15). However, in no study to date has a correlation between adduct levels and disease status been noted; that is, adduct levels are not significantly higher in the respiratory tract of lung cancer patients than in noncancer patients with the same level of tobacco smoke intake.

## DNA Adducts in Circulating Blood Cells

Several  $^{32}\text{P}$ -postlabeling studies have now been reported in which elevated levels of DNA adducts have been detected in blood cells of workers occupationally exposed to PAHs, and among whom there is a known or suspected increase in lung

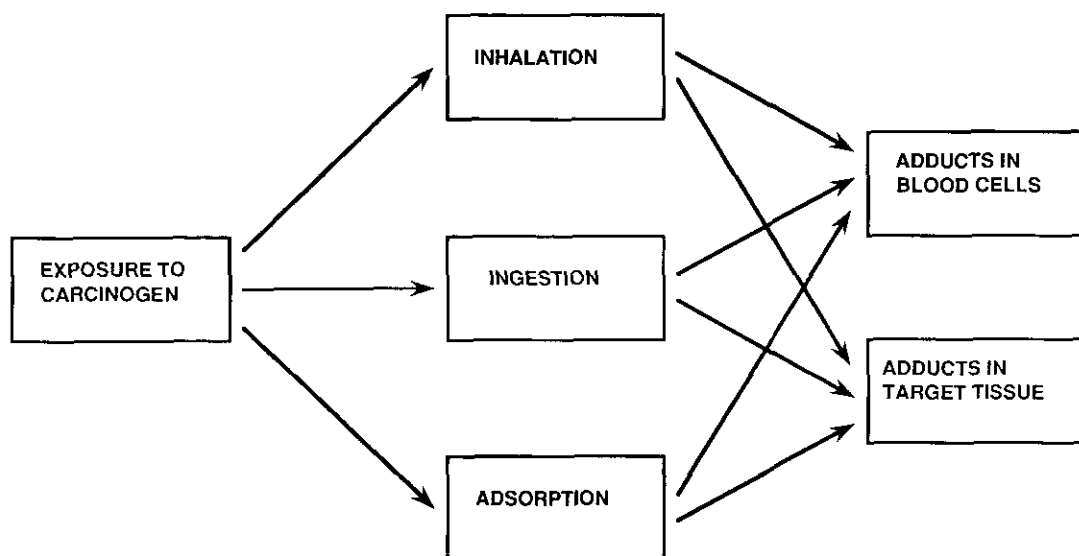


FIGURE 4. Schematic representation of the possibility of multiple routes of intake of carcinogens leading to adducts in tissues that are targets for tumor induction and in circulating white blood cells used as a readily obtainable surrogate source of DNA.

cancer incidence resulting from the exposure. Published studies now include data on workers in iron foundries (16), coke ovens (17), aluminium production (18), and roofers (19). All these studies clearly indicate exposure-related adducts in circulating blood cells, but it is interesting to examine the evidence relating to the influence of tobacco smoking on these adduct levels.

For many of these studies, elevated adduct levels due to smoking were not observed, although in some studies where lymphocytes were examined, rather than all white blood cells, a weak effect could be seen (18). Other studies that used matched groups of nonoccupationally exposed smokers and nonsmokers were also negative when whole white blood cells were analyzed (8,11), but positive in one of two studies in which lymphocytes only were examined (20,21). Analogous studies using methods of analysis other than  $^{32}\text{P}$ -postlabeling also give conflicting results: One study of coke oven workers using ELISA analysis of white blood cell DNA found an effect of smoking on adduct levels (22). Also, smokers with lung cancer were found to have higher adduct levels than smokers without cancer (23). However, in several other studies, no effect of smoking on adduct levels in white blood cells has been observed (17,24–28).

Thus, it would appear that the influence of tobacco smoking on adduct levels in circulating blood cells is no more than a weak one. As with the results with bladder DNA, the evidence points to the involvement of other sources of carcinogens, and whether or not a significant difference between smokers and nonsmokers is observed may depend on the strength of influence of these other factors in the particular populations being studied. Among occupationally exposed individuals, the occupational source of carcinogens appears to be the dominating influence in determining levels of DNA adducts in blood cells. Among nonoccupationally exposed individuals, sources of environmental genotoxic chemicals other than tobacco smoking make a greater contribution to the total DNA damage in white blood cells, and it is clearly important that more be learned about the origins of these adducts. The particular balance of intake of carcinogens by inhal-

ation, ingestion, and skin absorption (Fig. 4) will influence the distribution of adducts in different human tissues, and there is evidence that PAH-DNA adducts can arise in blood cells as a result of dietary intake (29–32). This and the possibility of skin absorption (33) will complicate attempts to extrapolate data on adduct levels in blood cells to levels in putative target organs.

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 26 October–1 November 1991.

The authors thank Greba O'Byrne for collecting tissues samples, Meadhbh Ni Shé for skilled technical assistance, and Fred Kadlubar for communicating his results to us prior to their publication. This work was supported by grants to the Institute of Cancer Research from the Cancer Research Campaign and the UK Medical Research Council.

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